EFFECTS OF ILLUMINATION AND TEMPERATURE ON METABOLIC PATTERNS OF THE ^{14}C–INCORPORATION BY THE MOSS, *DICRANUM SCOPARIUM*.[1, 2]

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Abstract. The moss, *Dicranum scoparium*, conditioned and maintained at temperatures ranging from 0° to 10°C, was subjected to acetate-2-^{14}C incubation at 5° and 22°C in the presence or absence of added illumination. Patterns of ^{14}C-incorporation into various non-protein fractions (including lipid-fatty acids) respired CO_2, and alcoholic solubles were examined. The ^{14}C-labeled metabolic products in amino acid-, organic acid-, and sugar-pools were separated and identified with column and thin-layer chromatographic techniques. Radioactivity increased in the sugar-pool under illumination regardless of incubation temperatures. The radioactivity in glucose remained unchanged regardless of illumination, but the formation of sucrose appeared to be light regulated. The combined regime of added illumination and raised temperature brought about an increase in activity for organic acid and a decrease in amino acid-pools although a sharp rise of ^{14}C-glutamine was occurring in the latter pool. High light gave rise to more ^{14}C-amino acids than those with a low light, regardless of incubation temperatures. Total level of activity in lipid-fatty acid pool remained unchanged during changes of illumination and incubation temperature, while its components, L_1 and L_2, fluctuated.

OHIO J. SCI. 76(3): 103, 1976.

Under laboratory conditions, metabolic patterns of higher plants can be modified by environmental variables, such as temperature and light. The accumulation of amides of amino acids is associated with temperature and light changes (Steward, 1963). Malic acid formation results from lowering of temperature (Vickery, 1954; Beevers *et al*, 1966) as well as from high light and stress temperature as observed by Brooking and Taylor (1973), using a sensitive grass, *Sorgham*. A change in incubation temperature of *Dicranum scoparium* brought qualitative changes of ^{14}C-incorporation in metabolic pools of the non-protein fraction (Wu, 1974). These changes in labeling were particularly noticeable among such individual compounds as aspartic, glutamic, malic, and citric acids; glutamine, glucose and two lipid-fatty acid components. The present study follows the ^{14}C-distribution pattern of *D. scoparium* under conditions of raised temperature as well as added illumination on various non-protein fractions.

[1]Manuscript submitted January 9, 1975 and in revised form January 10, 1976 (#75-2).

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METHODS AND MATERIALS

Radioactivity (gross and low relative) induced by added illumination on mosses incubated at 5 C, which was within its preconditioned temperature range (0°–10° C) was measured in two groups of mosses. One group was incubated under added illumination designated as "high light" (9.7 mW cm^{-2}) and compared with another group exposed only to laboratory ambient lighting (0.38 mW cm^{-2}) designated as "low light". The patterns of ^{14}C-acetate metabolism in mosses under illumination at raised temperature (22°C) were compared with mosses incubated in "high light" at 5°C, and with mosses incubated under "low light" as compared to "high light" conditions at 22°C.

The moss, *Dicranum scoparium* (Hedw.) which had been preconditioned and maintained at 0°–10°C in a growth chamber was used. Ten gametophytes were used in each incubation and duplicates were run for each determination. All chemicals including radioactive sodium acetate were similar to my earlier work (Wu, 1974) as were the precedures for incubation with ^{14}C-acetate as substrate at two temperatures, 5°C and 22°C. Illumination of 9.7 mW cm^{-2} was supplied from reflector bulbs

located beneath the water bath. Respired CO_2 was trapped in NaOH, and the non-protein fraction of the ^{14}C-plant specimens were collected at the end of each incubation. The non-protein fraction was further fractionated into lipid-fatty acid-, amino acid-, organic acid-, and sugar-pools using solvent solubility and column chromatographic procedures. Thin-layer chromatography was employed to resolve and to identify major ^{14}C-components in these metabolic pools. Radioactivity in each metabolic pool as well as in each isolated, major ^{14}C-component was determined in a Beckman liquid scintillation instrument.

Radioactivity (gross and relative) of each of the metabolic pools was determined and results were tested at the 90% confidence levels by t-test. Each metabolic pool was further analyzed with thin-layer chromatography (TLC), in order to isolate and identify major ^{14}C-components. This procedure provided patterns of labeling in each pool and indicated the extent to which specific compounds were involved in the metabolism of acetate-2-^{14}C by moss plants under different conditions. The ^{14}C-distribution data of one sample chosen from each treatment was done to show typical ^{14}C-spot patterns on the chromatograms of each pool; as revealed by repeat runs of TLC and radioautographs.

RESULTS AND DISCUSSION

Regardless of light conditions, there was significantly higher ($p<0.1$) ^{14}C-incoproration into both the alcoholic soluble and respired CO_2 fractions under high temperatures than under low temperatures (fig. 1). But there was a lower level of labeling under high light than under low light. My earlier observation (Wu, 1974) noted that with temperature as the sole variable, the increase and the decrease of ^{14}C-incorporation into alcoholic solubles of mosses followed the rise and fall of the incubation temperature. As demonstrated here, when a second variable, illumination, was introduced, the ^{14}C-incorporation was less at both incubation temperatures. This finding indicates either that temperature or light alone or that the combination of the two can affect the ^{14}C-incorporation into the alcoholic soluble fraction.

In the case of respired CO_2, the ^{14}C-incorporation into respired CO_2 was affected by high incubation temperature (22°C). It was shown also in my earlier observation (Wu, 1974) that a significantly higher ^{14}C-incorporation occurred at high incubation temperature than that at a low temperature. A significant reduction of ^{14}C-incorporation occurred during additional illumination at 22°C ($p<0.1$). However, there was no significant change at 5°C incubation temperature under either light condition, indicating that ^{14}C-labeling in respired CO_2 is affected by light only at a high incubation temperature (22°C) but not at low temperature.

Weigl *et al* (1951) demonstrated that the production of $^{14}CO_2$ by barley leaves was depressed by strong light. They suggested that there was a preferential utilization of respiratory $^{14}CO_2$ in photosynthesis. This refixation of $^{14}CO_2$ which resulted in a reduction in the amount of $^{14}CO_2$ evolved, may have happened in the mosses used in the present study, but, only at high temperature and not at low temperature.

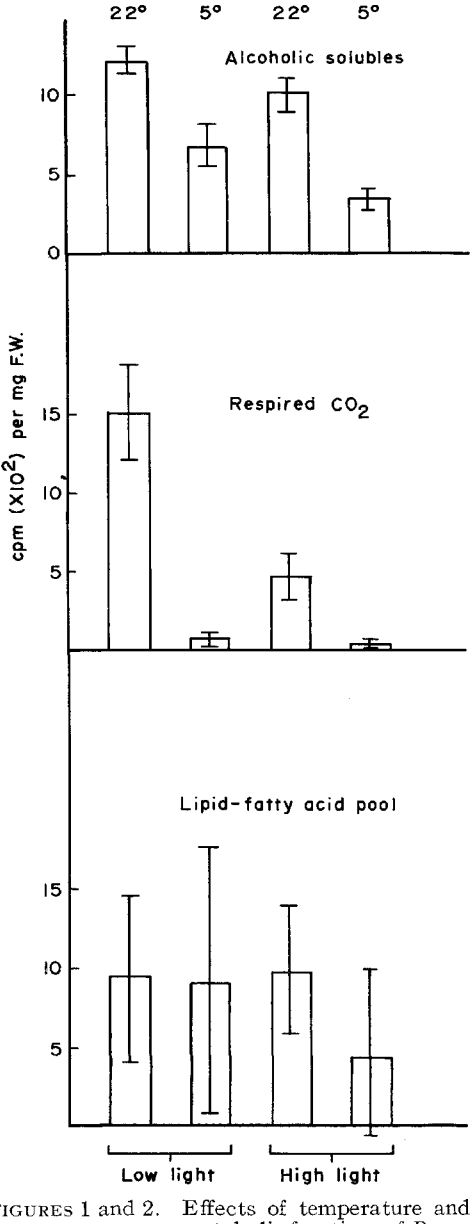
The ^{14}C-incorporation into lipid-fatty acid fractions was the same under all incubation conditions, indicating that neither temperature nor light had any marked effect on ^{14}C-incorporation into this metabolic pool. As shown in figure 2, the ^{14}C-incorporation distributed among the amino acid-, organic acid-, and sugar-pools (the alcoholic soluble fraction) were $> 50\%$, $< 50\%$, and $<2.0\%$, respectively. These pools were affected more by the combination of elevated temperature and high light than by elevated temperature under low light. Under the latter condition, there was no significant difference between the two incubation temperatures present in any of these three pools.

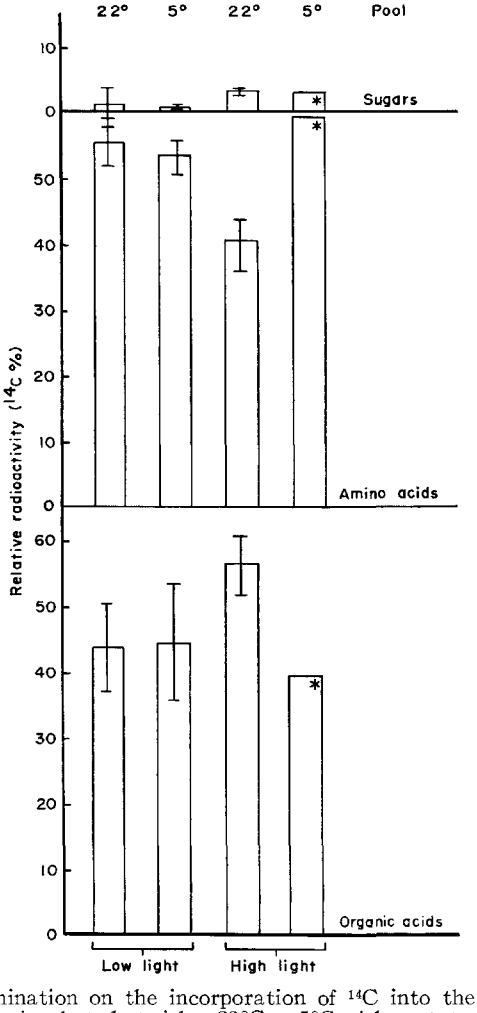
At both temperatures there was more ^{14}C-incorporation into the sugar pool under high light than under low light. This indicates that the ^{14}C-incorporation into sugars was affected by light regardless of temperature.

Under additional light (high light), a significantly lower ^{14}C-incorporation ($> 10\%$) occurred in amino acid pools at the 22°C incubation than those under low light; while at 5°C incubation the ^{14}C-labeling was higher in mosses under high light than in those under low light. Meanwhile, a significantly higher ($> 10\%$) ($p < 0.1$) ^{14}C-incorporation into the organic acid pool occurred at 22°C incubation while a lowered trend of ^{14}C-incorporation occurred with 5°C incubation. These data indicate that under

ambient light (low light) relative radioactivities among the three metabolic pools remained unchanged with varying incubation temperatures. On the other hand, additional light (high light) had a positive effect on ^{14}C-incorporation into sugars regardless of incubation temperature. High light had a negative effect on the amino acid-pool during high temperature and it brought about an increase in ^{14}C-incorporation in the organic acid-pool.

Following thin layer chromatographic and radioautographic analyses on the four metabolic pools—amino acids, organic acids, sugars and lipid-fatty acids, several compounds were found labeled; the extent and the appearance of labeling





FIGURES 1 and 2. Effects of temperature and illumination on the incorporation of ^{14}C into the metabolic fractions of *D. scoparium* incubated at either 22°C or 5°C with acetate-2-^{14}C. "Low light" denotes incubation under ambient lighting, (0.38 mW cm^{-2}), and "high light" denotes incubation under added illumination (9.7 mW cm^{-2}). Values are for gross or relative radioactivity. Vertical brackets show 90% confidence intervals as determined by the t-test. *No t-test used, due to loss of sample.

varied with different experimental conditions. Generally, higher levels of radioactivity were detected in each pool from mosses incubated at 22°C as compared with mosses incubated at 5°C.

Under ambient light (low light), at 5°C incubation, the major labeling (in terms of % ^{14}C-incorporation into each pool) was present in glucose (dextrose) (> 98%) of the sugar pool (figure 3); succinic-fumaric acids (< 10%), malic acid (15%), citric acid (70%) of the organic acid pool (figure 4). Two components, not yet identified, L_1 (> 70%) and L_2 (20%) of the lipid-fatty acid fraction (not shown); alanine (1.4%), aspartic acid (5.6%), glutamic-pyroglutamic acids (89%) and glutamine (1.4%) of the amino acid pool (not shown) showed labeling. When illumination (high light) was added to the same 5°C incubation there were increases in ^{14}C-incorporation present in the following: succinic-fumaric acids (> 20%), malic acid (30%); L_2 (30%), β-alanine (2.1%), leucine (3.3%), proline (2.0%); while decreases in ^{14}C-incorporation occurred in the others such as L_1 (< 70%), citric acid (< 40%), aspartic acid (nil), glutamic-pyroglutamic acids (66%), glutamine (nil), and relatively small or unchanged ^{14}C-labeling in all sugars that were isolated.

At 22°C incubation, under ambient light ^{14}C was incorporated mainly into glucose (> 80%) and fructose-mannose (< 20%) of the sugar pool. Succinic-fumaric acids (< 10%), malic acid (> 30%), citric acid (< 50%) of the organic acid-pool; L_1 (< 80%), L_2 (< 20%) of the lipid-fatty acid pool; alanine (1.0%), aspartic acid (12.7%), glutamic-pyroglutamic acid (82%) and glutamine (1.3%) of the amino acid-pool also showed incorporation. When added illumination (high light) was applied, an increase in ^{14}C-incorporation was found among: fumaric-succinic acid (> 10%), malic acid (> 40%), L_2 (> 30%); alanine (1.33%), glycine (0.53%), glutamine (9.7%) hydroxyproline (0.52%), proline (0.30%) and sucrose at 80%; while the major reduction of ^{14}C-incorporation oc-

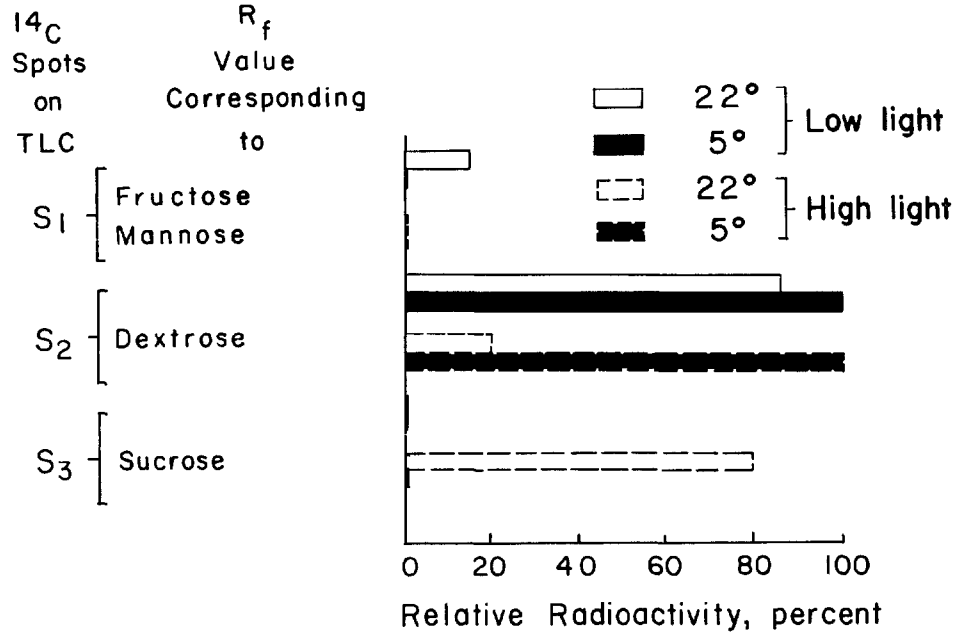


FIGURE 3. Effects of incubation temperature and illumination on the ^{14}C-incorporation from acetate-2-^{14}C into the organic acid pool of *D. scoparium*, as determined by thin-layer chromatography (TLC). "Low light" denotes incubation under ambient lighting (0.38 mW cm^{-2}) without added illumination. "High light" denotes incubation under added illumination (9.7 mW cm^{-2}).

curred in citric acid (< 40%), glucose (20%), fructose-mannose (nil), L_1 (60%), aspartic acid (8.5%), and glutamic-pyroglutamic acid (78%) (figs. 3, 4).

The bulk of radioactivity that was incorporated into the amino acid pool was present in glutamic-pyroglutamic acids. This activity was as high as 78–82%. This level remained relatively constant under all experimental conditions, except ^{14}C-incorporation was evident during high light indicating that the change in aspartic acid was brought about by light as well as by temperature. The rise and fall of ^{14}C-incorporation in aspartic acid was in the reverse direction with the ^{14}C-incorporation that occurred in malic acid under the similar conditions. Graham and Walker (1962) reported that with Mung bean, strong light led to

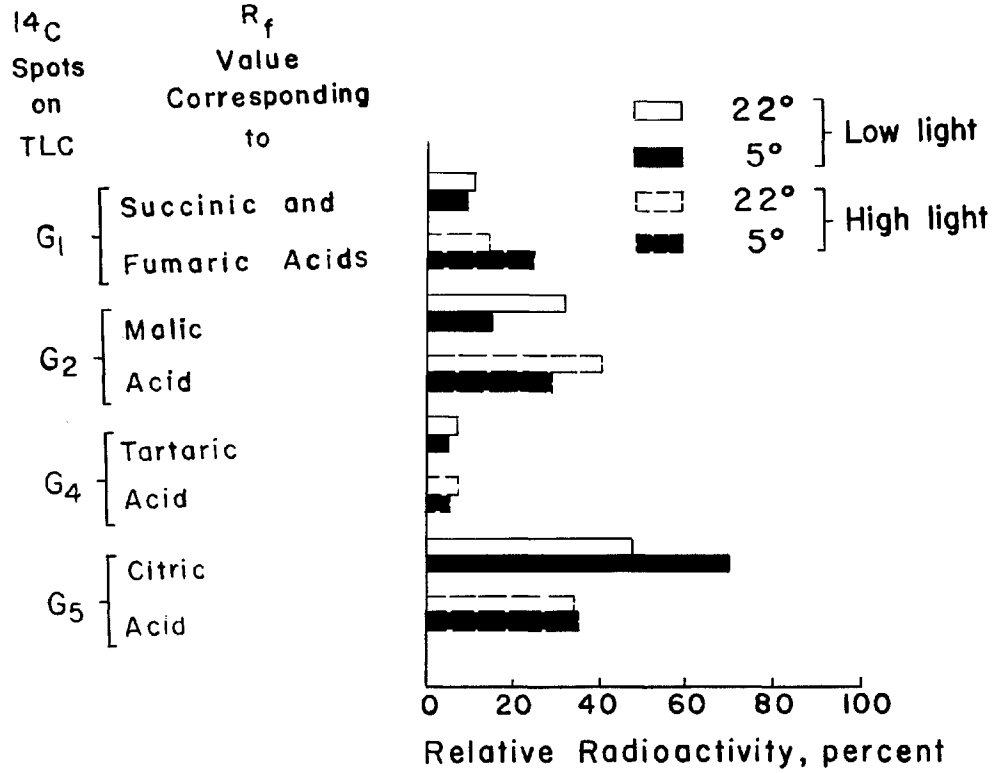


FIGURE 4. Effects of incubation temperature and illumination on the ^{14}C-incorporation from acetate-2-^{14}C into the sugar pool of *D. scoparium* as determined by thin-layer chromatography. "Low light" denotes incubation under ambient lighting (0.38 mW cm^{-2}) without added illumination. "High light" denotes incubation under added illumination (9.7 mW cm^{-2}).

at 5°C, where a drop of 30% labeling occurred when low light was changed to high light, indicating that the high light had an effect on glutamic acid synthesis in mosses at lower temperature but not at high temperature. Less labeling was found in aspartic acid at low incubation temperature (5°C) (Wu, 1974). This reduction was apparently enhanced by the effect of light as demonstrated in the present study whereby a lowering of a rise in malate and to a drop in aspartate, which resulted from a retardation in the conversion of malate to oxaloacetate in strong light. They interpreted this to indicate that there was less transamination occurring to produce aspartate while more malate was being synthesized. Concurrently, a lowering of ^{14}C-incorporation in citrate occurred as ^{14}C malate level increased under light especially at a raised incubation temperature indicating

a preferential synthesis of malate under light.

That additional illumination gave rise to labeled alanine, proline, leucine and arginine from acetate-2-^{14}C during 5°C incubation; and increasing ^{14}C-incorporation in glutamine and hydroxyproline during 22°C, is worth noting. Of these, the ^{14}C-labeling in arginine and glutamine was relatively high at 6.6% and 9.7% respectively. Changes in these two compounds may have been controlled by a system that was sensitized by low temperature as suggested by Sagisaka (1974), who used the poplar to show that glutamine contributed to normal growth while arginine contributed more to wintering. The labeled arginine arising from acetate metabolism during the low temperature plus high light condition of this study tends to support the suggestion that arginine is associated with cold temperature metabolism. Glutamine formation had been associated with light in mint leaves (Steward, 1963) and with raised temperature in mosses (Wu, 1974); the increase in ^{14}C-incorporation into glutamine during high temperature plus added illumination was to be expected.

There appeared to be a shift in activity (20%) from glucose to fructose and/or mannose, during low light for the 22°C incubation; and from glucose to sucrose (80%) during high light for the 22°C incubation. The appearance of the ^{14}C-labeling in sucrose supported the observation that accumulation of sucrose was associated with sunlight in mosses (Mason, 1916), and in stress tolerant ryegrass (Taylor *et al*, 1972). The same levels of radioactivity were incorporated into glucose during 5°C incubation under both light conditions, and almost all the activity resided in the glucose. These observations imply that glucose synthesis is closely involved in the utilization of acetate-2-^{14}C. It then can be mobilized to form other sugars, depending on the temperature and light conditions.

Although the total radioactivity incorporated into lipid-fatty acid fractions remained unchanged under conditions of elevated temperature and elevated light, the ^{14}C-incorporation into the two isolated components (presumably fatty acids) did change: L_1 decreased and L_2 increased. This finding indicated that both light and temperature (Wu, 1974) had effects on the ^{14}C-incorporation into individual components of this fatty acid pool, where a redistribution of ^{14}C-labeling might have resulted from a change of unsaturation of fatty acids. Such changes have been found not only during temperature change (Kuiper, 1970; Harris and James, 1969) but also under light change (James and Nichols, 1966).

From the results presented above, it is clear that the bulk of the assimilated carbon in *Dicranum scoparium* gametophytes distributed equally into the fat solubles (i.e. lipid-fatty acid fraction) and alcohol solubles (i.e. aqueous fraction). The ^{14}C-incorporation into the alcoholic solubles during acetate-2-^{14}C metabolism after incubation either at high (22°C) or low (5°C) temperature was essentially the same under ambient light (low light). Within the alcoholic solubles at least 98% of ^{14}C was in the form of amino acids and organic acids, with glutamic acid, aspartic acid, glutamine, malic acid and citric acids being the most heavily labeled. When added illumination (high light) was applied a considerable increase in ^{14}C-incorporation occurred in sugars and the ^{14}C-incorporation into amino acid- and organic acid-pools was also altered. Both light and temperature had a greater effect on ^{14}C-incorporation into individual compounds within each metabolic pool than on total ^{14}C relative levels incorporated into each pool.

Considering the amount of ^{14}C that was incorporated into the amino acid, organic acid, and lipid-fatty acid fractions from assimilated acetate-2-^{14}C, it would be advantageous to carry out further in-depth studies on these metabolic pools in mosses using acetate as ^{14}C substrate, for following the fate of the carbon in lower plants.

Acknowledgments. My thanks to Drs. J. R. Rastorfer and Emanuel D. Rudolph for their suggestions; to Drs. Edmund Schofield and Julia A. Schutte for helpful criticism of an earlier draft of this paper.

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